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Original Article

Exhaled nitric oxide as a marker of atopic asthma

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Abstract

Background: The aim of the present study was to assess the effect of atopy and regular therapy for asthma on exhaled nitric oxide (eNO).

Methods: Exhaled NO was measured using a chemiluminescence analyzer during slow expiration in 83 children, aged 5–7 years, after hospitalization for wheezing in infancy. The mean (±SD) age of the subjects was 7.2 ± 0.7 years, 28% were girls and 42% were atopic. A total of 31 children (37%) had asthma with regular medication: 20 were taking cromones, and 11 were taking inhaled steroids.

Results: In the asthma group, the median concentration of eNO was 14.9 p.p.b. (range 3.5–56.1 p.p.b.) in atopics and 7.3 p.p.b. (range 5.1–15.6 p.p.b.) in non-atopics ($P < 0.01$). The levels for atopic children on cromones tended to be higher than for those on inhaled steroids; however, the difference did not reach statistical significance.

Conclusion: We conclude that eNO concentrations associate significantly with atopic asthma.

Key words: asthma, atopy, children, cromone, exhaled nitric oxide, inhaled steroid, skin prick test.

Introduction

Exhaled nitric oxide (eNO) has been reported to be raised in children with asthma.[1–6] The probable explanation for this is an ongoing inflammation of the lower airways.[4,7] However, atopic and infectious manifestations of both upper and lower airways may also elevate eNO concentrations[5,8,9] and inhaled glucocorticosteroids,[2,10] as well systemic ones, may reduce it.[3,11] The measurement of eNO has been suggested to be a useful, non-invasive, easy-to-perform method to monitor asthmatic inflammation.[3,4,11]

Many studies have evaluated the role of eNO in monitoring the effectiveness of steroid medication in children with asthma but, in most studies, the number of patients has been rather low. The studies have offered evidence that inhaled steroids decrease eNO concentrations.[1,3,11–14] To our knowledge, only one report has suggested that regular cromone therapy reduces levels of eNO in children with asthma.[15] According to the international guidelines, cromones are recommended as first-line drugs for mild-to-moderate asthma in school-aged children.[16]

The aims of the present study were to examine eNO concentrations in children who had been treated in hospital for wheezing in early childhood, followed from infancy to school age, and to assess how atopy and regular therapy for asthma influence eNO.

Methods

Study design

A total of 95 children who had been treated in hospital for early childhood wheezing in over the period 1992–93[17] attended a follow-up visit in the Department of Paediatrics, Kuopio University Hospital, eastern Finland, in March 1999. At the study visit, eNO was measured and skin prick tests (SPT) were performed. The parents were advised not to give sympathomimetics to their children for 12 h preceding the eNO measurement.

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In addition, they were told that topical corticosteroids for the skin had to be avoided for 2 days, oral short-acting antihistamines had to be avoided for 3 days and oral long-acting antihistamines had to be avoided for 5 days prior to the investigation. The use of regular medication for asthma (i.e. inhaled steroids or cromones) was not restricted. Children who had had symptoms suggestive of respiratory infection during the preceding 2 weeks of the measurement were not allowed to participate in the study. Symptoms suggestive of allergic rhinitis, if present during the study, were recorded.

Detailed data of ongoing regular therapy for asthma were registered. The doses we usually prescribe for maintenance therapy for steady state asthma are 100–200 μg twice daily for inhaled steroids (budesonide and beclomethasone), 20 mg three times daily for cromolyn sodium and 6 mg twice daily for nedocromil. Among the 95 children studied, 12 used budesonide, two used beclomethasone, 15 used cromolyn sodium and seven used nedocromil. Budesonide was delivered by Turbuhaler® (AstraZeneca, Masala, Finland), beclomethasone by Easyhaler® (Orion, Espoo, Finland), cromolyn sodium by Eclipse® (Rhône-Poulenc Rorer, Helsinki, Finland), and nedocromil as an aerosol preparation by Syncroner® (Rhône-Poulenc Rorer).

Definition of asthma

Current asthma was considered to be present if either of the following criteria were fulfillled: (i) the patient was on continuous maintenance medication for asthma and had a history of at least three physician-confirmed wheezing episodes; or (ii) the patient had had a physician-confirmed wheezing episode during the preceding 12 months and had a history of at least two additional physician-confirmed wheezing episodes.[16] During the follow-up visit, asthma was divided into five categories on the basis of recent symptoms[18] assessed clinically by one of the authors (MK). According to our guidelines, children with mild-to-moderate asthma are treated with cromones and those with severe symptoms or those not responding to cromone therapy are treated with inhaled steroids.[19]

Pulmonary function testing

The baseline pulmonary function was examined by flow-volume spirometry (Medikro, Kuopio, Finland), and forced expiratory volume in 1 s (FEV_1) was the parameter used to assess the degree of obstruction. The result of at least 75% of that of predicted was regarded as a normal.[19] First, the children were carefully instructed on how to perform the test. Thereafter, measurements were repeated at least three times and accepted if the FEV_1 variation was less than 5% and the printed graphic curves were appropriate and equal in shape. The highest FEV_1 value was selected.

Measurement of eNO

The eNO concentrations were measured using a chemiluminescence analyzer (NOA 280; Sievers Instruments, Boulder, CO, USA).[20] Subjects were asked to avoid physical exercise for at least 15 min prior to the measurement. During measurement, subjects were seated while slowly exhaling from total lung capacity for a 20–30 s period against a fixed expiratory resistance (20 mmHg), by closing the velum of the soft palate, to eliminate contamination with nasal NO. A biofeedback exhalation flow display provided visual guidance to help the subject maintain their exhalation flow at the desired level of 50 mL/s. Exhaled air was led through a norebreathing valve into a Teflon tubing system connected to the analyzer. Recordings were performed by a single-breath program (Sievers Restricted Exhaled Breath Program 2.01; Sievers Instruments). Two successive exhaled samples were obtained from all subjects.[4,21] The relative standard deviation between the samples was expected to be below 10%, with results being expressed as mean values, using a unit of parts per billion (p.p.b). The measurements were performed in the same laboratory under constant conditions under the supervision of one of the authors (KK) and were stored digitally on the computer. Readings from the stored data were made by another investigator (MP) who was unaware of either clinical data or the results of the SPT of the study subjects. The chemiluminescence analyzer was calibrated daily using a zero air and a certified concentration of NO.

Skin prick testing

Skin prick tests were performed for all children. The allergens (ALK skin prick test extracts; ALK Laboratories, Copenhagen, Denmark) tested were the outdoor allergens birch, common alder, timothy grass, meadow grass and mugwort pollens and spores of *Cladosporium herbarum*. The indoor allergens tested were cat and dog epithelial danders and home dust mites *Dermatophagoides pteronyssimus* and *Dermatophagoides farinae*. The concentration of the non-standardized allergen extract, *C. herbarum* spore, was 1 : 20 weight/volume.

Other allergen extracts were standardized, the concentrations being 10 histamine equivalent points. Histamine hydrochloride (10 mg/mL) was used as a positive control and 50% glycerol was used as a negative control. Wheals with a median diameter of at least 3 mm were regarded as positive[22] and no reactions were allowed in negative controls. A child was defined to be atopic if there was at least one positive result in the SPT.

Patients

The eNO results of nine of 95 children were excluded from analysis because of acute respiratory infection in four cases, poor repeatability of the successive measurements in three cases and insufficient cooperation in two cases. Data of three non-treated children with asthma are presented separately and are not included in the statistical analyses. The remaining 83 children, with a mean (±SD) age of 7.2 ± 0.7 years, were included in the analyses; 28% were girls, 42% were atopic and 37% had asthma. Only two children had symptoms suggestive of allergic rhinitis at the time of the measurement. All but the three children with asthma were taking regular medication for asthma: cromones in 20 cases (59%) and inhaled steroids in 11 cases (32%).

Ethics

The study design was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the parents of the children.

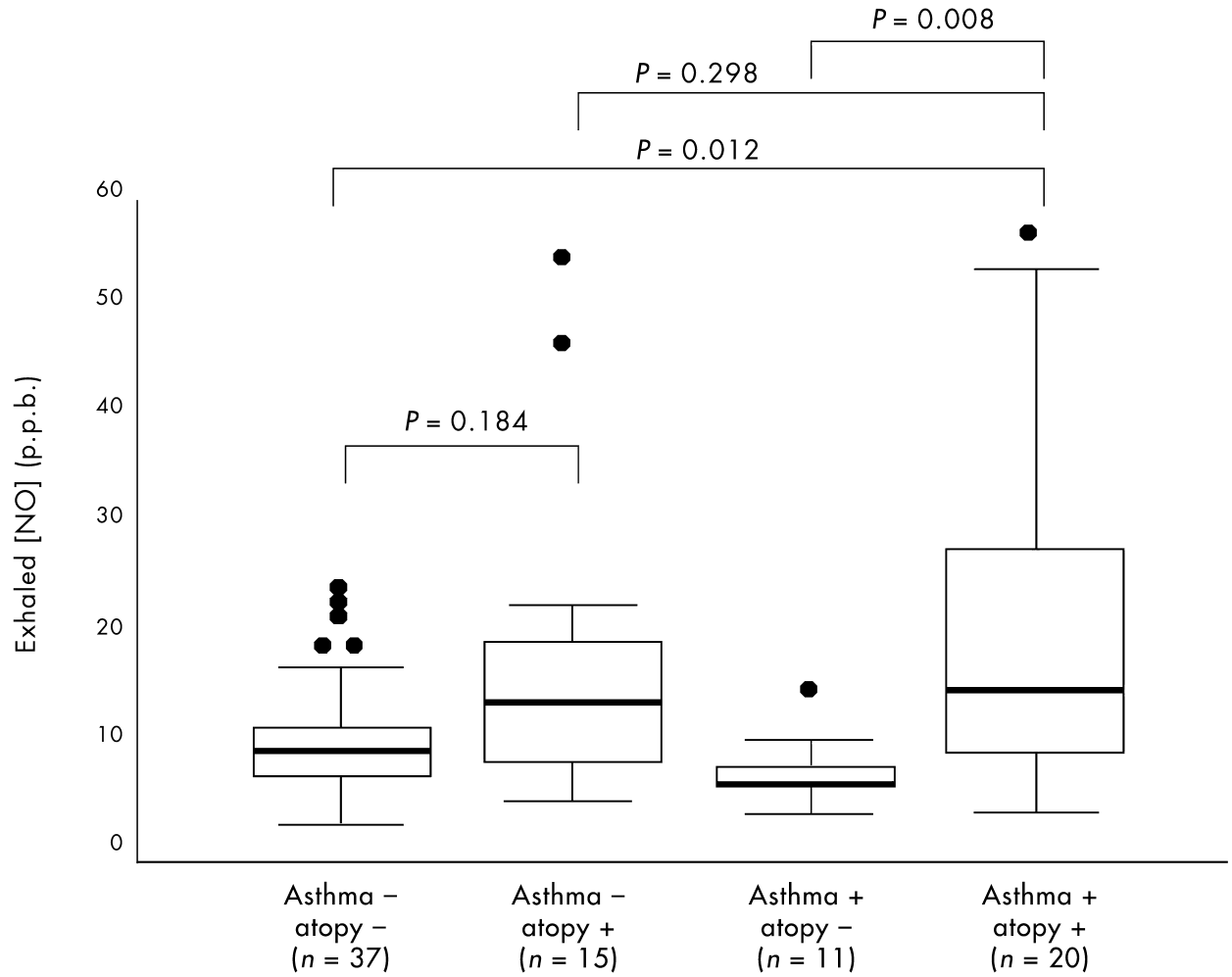

Fig. 1 Levels of exhaled nitric oxide (NO) for 31 children with asthma and for 52 children with no asthma, presented in relation to atopy. The 31 children with asthma were on regular medication. The line in each box is the median; box limits represent the 25th and 75th percentiles; the whiskers extend to the 5th and 95th percentiles. Values above the 95th percentile are plotted separately. The two atopic, non-asthmatic outliers are excluded from the statistical analyses (see text).

Statistics

Data were analyzed using SPSS/PC+9.0 software (SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov test was applied to assess the normality of the distributions of continuous variables. Because the data for eNO did not follow a normal distribution, not even after logarithmic transformation, a non-parametric Mann–Whitney *U*-test was used in comparisons between groups. Fisher's exact test was used for 2 × 2 contingency tables. A general linear model, applying natural logarithmic transformed data, was used to assess both the main and interaction effects of confounding factors on the levels of eNO. Skin prick test reactivity, asthma and sex were the variables included in the model.

Results

The mean (±SD) age of the 83 study subjects was 7.2 ± 0.7 years, 60 (72%) were male and 31 (37%) had medicated asthma. The baseline FEV_1 was normal for every child included in the analysis. The children were classified into four categories on the basis of the presence of atopy and asthma (Fig. 1). As seen in Fig. 1, there were two atopic, non-asthmatic children with exceptionally high eNO values over 45 p.p.b. These children were regarded as outliers[23] and they were excluded from further analyses. In fact, they had had subjective symptoms suggestive for asthma (e.g. prolonged cough), but they did not fulfill the objective criteria for asthma. The eNO values were higher among the atopic than non-atopic children. The difference was significant in the asthma ($P < 0.01$) but not in the non-asthma group. The distribution of eNO values was especially wide and skewed to the right if both asthma and atopy were present.

The effects of atopy, asthma and sex on eNO were studied by including them in the general linear model. The children were rather homogeneous in age, which was not considered a confounding factor and was not

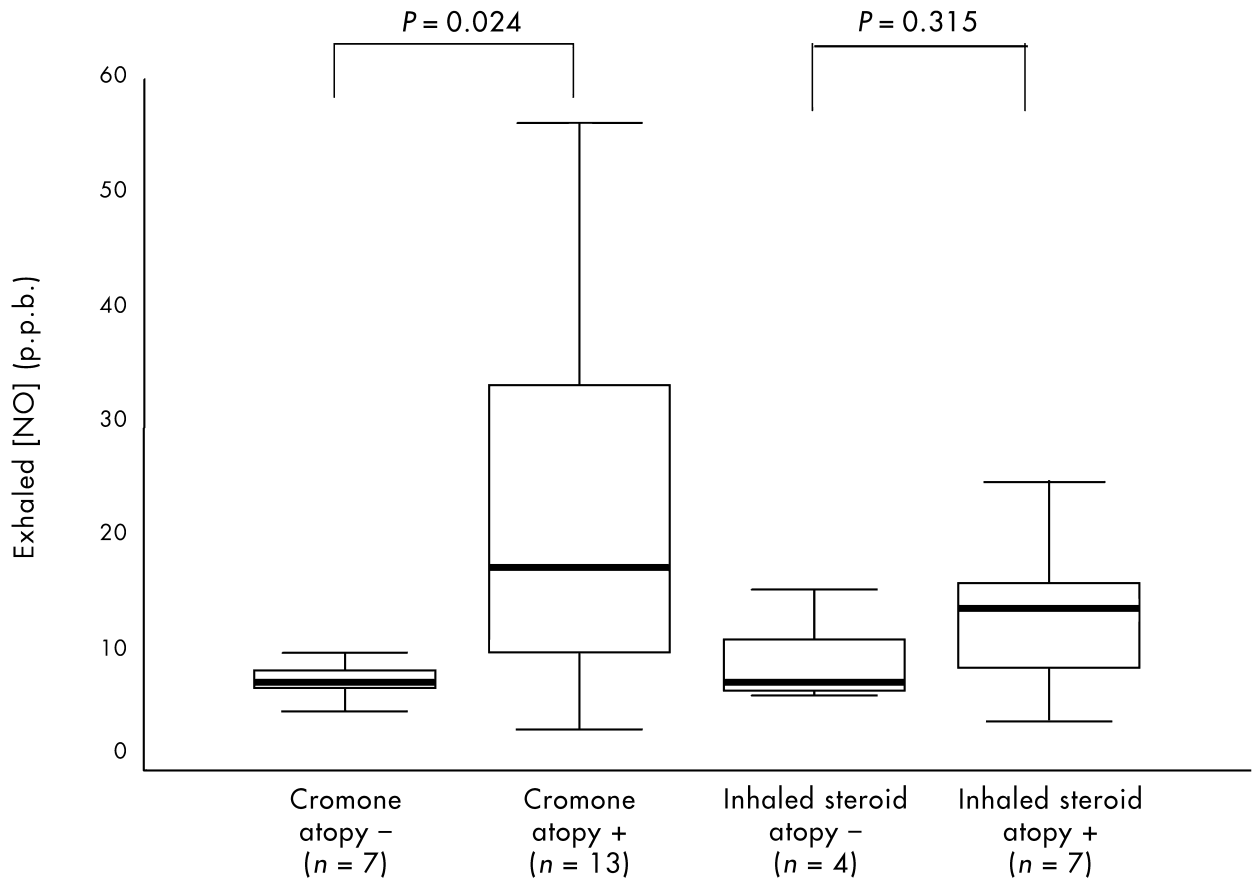

Fig. 2 Levels of exhaled nitric oxide (NO) for 20 children with asthma treated by cromones and for 11 children treated by inhaled steroids, presented in relation to atopy. The line in each box is the median; box limits represent the 25th and 75th percentiles; the whiskers extend to the 5th and 95th percentile.

included in the model. Atopy, defined as one or more positive reactions in the SPT, was the only factor that had a significant association ($P < 0.01$). No interactions between atopy, asthma and sex were found. Among the children with asthma on regular medication, 23 of 31 had mild symptoms. There were only three children with no symptoms and five had moderate or severe symptoms; these figures were too small for any statistical calculations.

The effects of treatment strategy on eNO were analyzed separately for the asthma group (Fig. 2). Among the 20 children on cromones, eNO values were significantly higher for atopic than non-atopic children ($P < 0.05$). A similar trend, although not statistically significant, was seen among children on inhaled steroids. However, eNO did not differ between children on cromones and those on inhaled steroids. The result was the same whether or not it was adjusted for atopy.

The 95th percentiles for eNO concentrations were calculated for children with no asthma, separately for atopic and non-atopic children. Among the non-atopic children, the limit was 16.8 p.p.b. and it was exceeded in 0 of 11 cases if asthma was present and in five of 37 cases (14%) if it was not (NS). Among the atopic children, the respective limit was 22.9 p.p.b. and it was exceeded in seven of 20 cases (35%) if asthma was present and in two of 15 cases (13%) if it was not (NS).

The three children with non-treated asthma were boys, aged 5.9–7.1 years. Two had positive SPT reactions to indoor allergens and none reacted positively to outdoor allergens. The eNO values were 46.5 and 7.0 p.p.b. for the two atopic children and 11.5 p.p.b. for the non-atopic child.

Discussion

In agreement with earlier reports, high eNO values were significantly associated with atopy.[5,8] We defined atopy on the basis of skin test results. This is an objective way to screen atopics from non-atopics, especially if anti-inflammatory medication is used. Elevated levels of eNO have also been reported in children with non-treated asthma.[6] When atopy has been taken into account, high eNO levels have been reported among children with atopic asthma, but not among those with non-atopic asthma.[5] In the present study, all but three children with asthma were on regular medication and asthma alone was not significantly associated with high eNO. This may reflect the efficacy of the regular therapy. In contrast, the 95th percentile for atopic children with asthma was exceeded by one-third of cases.

The expression of an enzyme responsible for the production of NO, inducible NO synthase (iNOS), is inhibited by glucocorticosteroids.[24] Byrnes *et al*. found that eNO was higher among children with asthma on bronchodilator therapy than among those on regular inhaled steroids. Moreover, it has been shown that eNO is reduced after commencement of inhaled steroid treatment.[2,10,12,14] Interestingly, in a Swedish study, eNO remained higher than normal in atopic children even though their asthma was treated with inhaled steroids and was considered as stable.[4] To our knowledge, only one report has suggested that cromones also reduce eNO in children.[15] A limitation of that study was the very small number of children on cromones (only five subjects). We followed 83 children from infancy to school age and 20 of them were on cromones at the follow-up visit. Our results suggest that cromones may not reduce eNO as effectively as inhaled steroids. However, no statistical difference in eNO was found between these two medication groups, in all probability owing to the small number of steroid-treated subjects.

Most children from the age of 7 years onwards are able to achieve and maintain a steady flow during eNO measurements.[3,8] Our experiences are in accordance with these findings: after careful instruction, reliable measurement of eNO failed in only 5% of children.

There are three strengths to our study. First, the cohort was rather homogeneous: the average age variation of the subjects was less than 1 year. Second, we have followed up the children from infancy, so their asthma diagnoses are based on long-term prospective follow up. Third, the study was performed outside the pollen seasons, which are known to affect results.[25] However, two atopic children on cromones had symptoms suggestive of both asthma and allergic rhinitis at the time of the eNO measurement and both of these had elevated eNO levels. In addition, to maximize the objectivity of the results, different physicians were responsible for prescription and follow up of regular therapy for asthma, for clinical definition of current asthma, for performance of the tests and for the interpretation of the results.

Only three children with current asthma had no regular medication, so statistical analyses were not possible for them. There are three other minor shortcomings in the present study. First, because the cohort consisted of children admitted to hospital for wheezing in early childhood,

all subjects had wheezed at least once, so there was no optimal control group. Second, there were many subgroups in relation to asthma and atopy in the cohort, leading to small numbers of cases and to low powers in statistical analyses. Third, the distributions of eNO levels were skewed. The majority of values were low, allowing patient-specific evaluation of the high values.

In conclusion, eNO concentrations associate with atopic asthma. The results were rather similar in children with regular therapy for asthma and in those with no asthma, reflecting the effectiveness of anti-inflammatory therapy.

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